PART III

CHAPTER 17

STRESS, HORMONES AND MORTALITY IN SMALL CARNIVOROUS MARSUPIALS

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This chapter covers some of the basic ecological, behavioural and physiological changes that have been reported in studies of dasyurid marsupials exhibiting the unusual post-mating male mortality (life-history Strategy I). Rather than attempting to cover all reports, the chapter concentrates upon changes that the author considers to be most relevant in attempting to explain why post-reproductive males in the wild die, yet females survive. Evidence relating to a defect in feedback of corticosteroid hormones in discussed in relation to recent findings for eutherian stress models. The relevance of these studies for explaining the truncated lifespan of Strategy I dasyurid males is discussed and an integrated flow diagram is attempted to synthesise the physiological changes that are likely to occur in the weeks preceding the death of males. Considered together, the adaptive physiological changes that occur during the last few weeks of life of the males are remarkable in enabling them to maximise their reproductive potential prior to a rapid physiological decline that involves stress related dysfunction and pathologies involving renal, gastrointestinal (GI), neuroendocrine and central nervous systems.

INTRODUCTION

While the dramatic post-spawning mortality of Pacific salmon is well known, one of the most remarkable phenomena to occur in the animal kingdom is the annual post-mating death of males that occurs in several species of small dasyurid marsupials in Australia and South America.

Various studies have been carried out during the past 30 years describing the occurrence in several species of small dasyurid marsupials of an annual post-mating mortality of the males (see Woolley 1966; Lee and Cockburn 1985; Lee and McDonald 1985; Cockburn 1989; Tyndale-Biscoe and Renfree 1987). Krajewski, Woolley and Westerman (2000) provide a comprehensive reference list of these small dasyurid species that exhibit the Strategy I life-history pattern (Lee, Woolley and Braithwaite 1982). This is characterised by a monoestrous reproductive pattern, male maturity at 11 months and the disappearance of males from the population within two to three weeks of the commencement of mating. Reproductive strategies in dasyurid marsupials, and the implications of molecular phylogeny, are also treated very comprehensively by Krajewski et al. (2000).

Recent taxonomic reassessments of genus *Antechinus* are relevant to several earlier studies of dasyurid marsupials in Eastern and SE Australia. Studies of *A. stuartii* carried out at Mount Glorious in

SE Queensland would now be regarded as studies of *A. subtropicus* (Van Dyck and Crowther 2000) while studies of *A. stuartii* from SE Australia, such as those ecophysiological studies conducted near Warburton (Bradley et al. 1980), would now be regarded as investigations of *A. agilis* in the light of reassessments by Dickman et al. (1998) and Sumner and Dickman (1998). Behavioural studies carried out on *A. stuartii* in forests near Canberra would be regarded as studies of *A. flavipes* (Sumner and Dickman 1998). To avoid confusion in references to *Antechinus* in this chapter, the new species names will be included in brackets after the species names used in the original citation.

Braithwaite and Lee (1979) observed that the species exhibiting the phenomenon are restricted to coastal regions of Australia where environments are predictable and seasonal. They considered a predictable environment to be important to provide resources for reproduction in species where a prolonged period was required to raise offspring from conception to weaning. Because only one reproductive period was possible under these conditions, there were benefits in investing heavily in their first bout of reproduction. This argument has been extended to provide an explicit physiological model for die-off in *Antechinus* (Lee and Cockburn 1985a, b; Lazenby-Cohen and Cockburn 1988; Cockburn 1992, 1997).

Studies on *Phascogale tapoatafa* (Cuttle 1978, 1982; Soderquist 1993; Soderquist and Ealey 1994) suggest that as with a number of *Antechinus* species, all males die at the conclusion of their first breeding season. Furthermore, Dickman and Braithwaite (1992) have described a post-mating mortality of males in the dasyurid marsupials, *Dasyurus hallucatus* and *Parantechinus apicalis*, the former being the largest dasyurid species to show this unusual phenomenon.

This life-history pattern also occurs in two South American marsupials, *Monodelphis dimidiata* (Pine 1994) and the mouse opossum *Marmosa incana* (Lorini et al. 1994). This life-history pattern has been classified as Strategy 1 (Lee, Woolley and Braithwaite 1982). Examination of West Australian Museum records for *Phascogale calura* in 1980 (Bradley unpublished) indicated that no adult males persisted in the records after the reproductive period, suggesting a post-mating male mortality did occur. This was reported by Kitchener (1981) and subsequently confirmed in field studies (Bradley 1982a; 1990a, b; 1995; 1997). The phenomenon was commented upon by Lee et al. (1982) and Lee and Cockburn (1985).

The Strategy I life-history pattern also occurs in some salmon species in which the death of males closely follows spawning. In these salmon species, an elevation in plasma cortisol concentration has been implicated in the stress related demise of males. In the Strategy I dasyurid males, during the mating period, there also occurs a marked elevation in plasma cortisol concentration however a concurrent rise in plasma testosterone and an androgen induced decrease in the plasma concentration of corticosteroid binding globulin (CBG) results in a marked increase in free (biologically active) cortisol.

This disappearance of males from the population at the end of their first breeding season (Strategy I males) has been shown to be a consequence of stress characterised by an increase in the plasma glucocorticoid (GC) concentration (Barnett 1973; Bradley, McDonald and Lee 1975; Lee, Bradley and Braithwaite 1977; Bradley and Monamy 1991) and exacerbated by an androgen dependent decrease in plasma CBG concentration (Bradley, Mc Donald and Lee 1980; McDonald et al. 1981; Bradley 1987). The phenomenon has been related to negative nitrogen balance (Woollard 1971), anaemia (Cheal et al. 1976; Bradley 1990a), GI haemorrhage and disease (Moore 1974; Bradley 1977, 1985, 1987; Barker et al. 1978; Bradley et al. 1980) and immune suppression and disease (Cheal et al. 1976; Bradley 1977, 1987; Barker et al. 1978, 1991; Bradley et al. 1980; see also review by Lee and McDonald 1985), and to unspecified degeneration of major organs (Williams and Williams 1982). McAllan, Roberts and O'Shea (1996) have also implicated dramatic changes in renal morphology of males in the male mortality.

Wexler and Greenberg (1978) described, in male Sprague-Dawley rats kept in active stud service, the development of abnormal metabolic and pathophysiological changes, many of these involving the cardiovascular system. Furthermore, studies by Von Holst (1972; 1986) have clearly shown the pathophysiological effects of stress in tree shrews *Tupaia belangeri* and in this species renal failure has been reported to be a major cause of death.

QUESTIONS

A. subtropicus males and females captured three months before the natural disappearance of males in the wild and treated to remove ecto-and endo-parasites have survived for up to five years (authors observations), which is in striking contrast with the natural lifespan of approximately 11.5 months for males. How might we explain this difference between realised and potential longevity of males? Why is such a high concentration of free cortisol in the plasma tolerated in males when feedback mechanisms might be expected to terminate the short-term response and allow the plasma free cortisol concentration to return to baseline levels? What is the proximate cause of death and how might this be related to the physiological changes that occur as part of this unusual life-history strategy?

These are but some of the interesting questions that may be raised in respect of the life-history of Strategy I dasyurid marsupial males.

PATHOGENESIS OF GASTRIC ULCERS

A significant advance in our knowledge of the pathogenesis of gastric ulcer in man has been the recognition that the bacteria, *Helicobacter pylori*, was causally related to the development of gastritis and peptic ulceration (see Marshall, McCallum and Guerrant 1991). In pursuit of a bacterial aetiology for GI ulcers in Strategy I dasyurids, numerous samples of gastric tissue from *A. flavipes*, *A. agilis* and *A. subtropicus* were examined histologically using the Warthin-Starry and Giemsa staining methods. The bacterium was not identified in any of the tissue samples and it seems that a causal association between *Helicobacter* sp. and the pathogenesis of gastric ulcers in these marsupials is unlikely.

Most authors now agree that GU pathogenesis is an example of a multifactorial disease process and many consider the disease to have a 'bio-psychosocial' origin (Leverstein 1998; Overmier and Murison 2000) with a combination of biological and psychological or social factors contributing to GI pathology.

Clinicians have reported that in both human and animal studies, high does of exogenous GCs increase the incidence of gastric ulceration (Bradley et al. 1975; Messner et al. 1983) and based upon this notion it was concluded that the increased release of exogenous GCs observed during the stress response was also ulcerogenic. Indeed, the comparison of experimentally stressed and non-stressed animals will often result in a significant correlation between endogenous steroid production and stress ulceration (Murphy et al. 1979; Filaretova et al. 1999).

While most authors agree that GCs do not have a direct effect upon the gastric mucosa (Kuwayama et al. 1989) there are many views about the ways in which indirect links may operate. GCs may inhibit proliferative repair in *A. flavipes* (Bradley and Richardson unpubl. obs.) and gastric mucosal microcirculation may be compromised (Kuwayama et al. 1989; Caparni de Kaski et al. 1995). The gastric microcirculation is a primary factor in the maintenance of gastric mucosal integrity, and in the protection of the mucosa from *H. pylori*, non-steroidal anti-inflammatory agents, and stress.

Endoscopic studies of GUs carried out on human patients indicate that mucosal blood flow is decreased in nearly all regions of the stomach during the active ulcer stage of the disease (Kawano et al. 1991) compared to normal controls. In contrast, during the ulcer healing stage the blood flow in the area of the ulcer increases markedly. These authors also noted that intractable ulcers, which did not heal within three months of treatment, showed virtually no increase in mucosal blood flow in the ulcerated region. Thus a decrease in the microcirculatory capacity of the stomach would appear to contribute to the development of gastric ulceration, whilst a return to normal levels of blood supply is clearly implicated in ulcer repair.

Decreased mucosal blood flow is a typical aspect of the GI stress response (Svanes et al. 1984). Rats exposed to a water-restraint stress regime are reported to experience a decrease in blood velocity in the submucosal microvessels, accompanied by the appearance of gastric erosions (Filaretova et al. 1999). Curiously it was found in this study that GC deficiency exacerbated the effects of stress on the microcirculation and that GC replacement eliminated the effects of GC deficiency, implying that GC release during stress may have a role in maintaining gastric microcirculation. The effects of stress on the normal structure and function of the gastric microcirculation clearly warrant further investigation.

Because of the ethical issues surrounding the induction of GUs in humans, animal models have greatly aided in our understanding of the pathophysiology of GU disease. A criticism of many earlier studies is that they employed methods to induce ulceration that were unrelated to the natural experience of the animal species under investigation. Some recent studies have recognised this problem and used more naturalistic animal models that allow for experimental analysis of the aetiology and symptomatology of ulcer disease that is more applicable to human GU research (Koolhaas et al. 1997).

During the stress response involving the HPA axis, stimulation of the sympathetic nervous system, a finding that would be expected as part of the stress response, would also contribute to gastric mucosal ischemia. GCs that are clearly elevated during the last weeks of life of Strategy I males are known, in eutherian species, to have a permissive effect that assists catecholamines and other vasoconstrictors to exert their full actions (Bondy 1981; Krakoff 1988).

Haemorrhage from gastric ulcers has been reported in several non-human species. While gastric ulcers do appear in natural populations of wild mammals, death is not the only consequence. Evidence of this was provided by Stemmerman and Hayashi (1969) who described the finding of healing gastric ulcers in individuals in a wild population of Hawaiian Feral Mongoose. Several reports exist for the occurrence of GI ulcers and haemorrhage within the Marsupialia. These include *Sarcophilus harrisii* (Hamerton 1935, 1938) various macropodid marsupials (Hamerton 1934, 1935, 1938, 1939) and *Didelphis virginiana* (Sherwood et al. 1968).

In spite of the current thinking about the absence of a direct link between GCs and gastric ulcer in man, the situation in small marsupials is not so clear. Studies on the pathogenesis, prevention and treatment of gastric ulcer have been examined in a range of mammals that include rodents, lagomorphs, primates and carnivores (Harding and Morris 1977; Kitagawa, Fujiwara and Osumi 1979; Hosoda, Ikedo and Saito 1981; Kivilaakso et al. 1981). These studies, and those carried out in more recent years, indicate that the pathogenesis of stress ulcers is multifac-

torial and may involve vascular, neurogenic, and central nervous system (CNS) mechanisms (Hernandez 1990).

An obvious question to ask is what evidence exists to support a direct link between glucocorticoid concentration and gastric ulceration in these species. The evidence for a direct link is equivocal. The administration of glucocorticoid for a prolonged period in male *A. stuartii* caused a dose-related mortality (Bradley et al. 1975) with haemorrhage from gastric ulcers being the most common cause of death. However another cause of death was the development of liver abscesses resulting from *Listeria monocytogenes* infection that in turn was most likely aggravated by immunosuppressive effect of the GCs. The administration of GCs to *A. flavipes* (Bradley and Richardson unpublished) resulted in a higher mortality in the glucocorticoid treated groups than in the controls, however the result was again rather equivocal. The GC caused a dose-related decrease in the rate of gastric-cell renewal determined by 3H-thymidine labelling followed by autoradiography (Martin and Menguy 1970). In Strategy I males, starvation plus sympathetic nervous induced gastric mucosal ischemia could effectively combine to threaten both gastric mucosal function and structure, and to compromise repair.

While an increase in parasite/microbiological activity associated with glucocorticoid induced immune suppression in these males has been discounted as the proximate cause of death (Lee et al. 1977), haemorrhagic peptic ulcers seem ubiquitous. In the Strategy I species in which the gastric mucosa has been examined almost all terminal males show some evidence of ulcers, often with evidence of associated acute bleeding. An almost universal finding in post-reproductive males that are found either in a debilitated or hypothermic condition, is the presence of haemorrhagic ulcers (Bradley 1977; Barker et al. 1978; Bradley McDonald and Lee 1980; Bradley 1987; Bradley 1997). While the pathogenesis of these gastric ulcers is unclear, high cortisol concentrations can be shown to reduce the rate at which gastric cell renewal can take place (Bradley and Richardson unpublished) and short periods of hypothermia can predispose males on a restricted food intake to the development of haemorrhagic ulcers (Bradley unpublished).

This observation from studies with rats fits quite well into a model that I envisage to describe the pathogenesis of gastric ulcers in small Strategy I dasyurid marsupials. It is well known that males captured in the wild within about two weeks of the disappearance of males are unlikely to survive, even if housed alone in a quiet environment with adequate shelter, food and water. Females, on the other hand, generally survive with losses being uncommon. Necropsies carried out on males that die invariably reveal extensive haemorrhaging from gastric ulcers. These present as diffuse surface erosions that do not appear to involve larger blood vessels in the adventitia and do not result in perforation of the stomach wall. It is suggested that males, near the time of their disappearance from the natural population, are practising 'physiological brinkmanship' and that the imposition of an additional stressor such as handling, captivity, mild hypothermia or even a threatening olfactory experience (Toftegaard et al. 1999, 2002), may be sufficient to stimulate the stress response and compromise the integrity of an already susceptible gastric mucosa.

Studies of larger dasyurid marsupial species (Dickman and Braithwaite 1992; Oakwood 1999; Oakwood et al. 2001), suggest that life-history variation in dasyurid marsupials is more common than has been previously suspected. My own observations of the GI tract of males of these larger dasyurid marsupials at necropsy is that the incidence of haemorrhage from gastric ulcers is common. Perhaps a larger body mass means that insidious blood loss from a GI lesion is less likely to be lethal and the extra energy reserves available to an animal of larger body mass might become significant for repair and survival. Furthermore, hypothermia would be less likely to develop as body mass increases.

Social stressors have been implicated in combination with hypothermia and restricted diet in the formation of GI lesions (Filaretova et al. 1998). While psychogenic stressors such as restraint are known to promote ulcer formation in other small mammal models (Filaretova et al. 1998) it is suggested that social stressors may be very significant in dasyurid marsupials and, when combined with hypothermia and restricted diet, form a potent ulcerogenic combination. The semiochemical 2,6-dimethylpyrazine, present in the urine of intact *A. subtropicus*, (Toftegaard, Moore and Bradley 1999) is a potent stressor for other males, elevating both plasma cortisol and catecholamines (Toftegaard 1999; Toftegaard and Bradley, in prep; see chapter by Toftegaard and Bradley). The increase in density suggested in Fig. 1 reflects a likely increase in *apparent density* that would be experienced by males during the reproductive period as they move around and deposit urine containing androgen dependent semiochemicals such as 2,6-dimethylpyrazine.

In a study of the pathogenesis of gastric ulceration in *A. subtropicus* (Hanlon 2001) reported that an elevation of free cortisol, combined with elevated testosterone, hypothermia and a psychological stressor (restraint), has a profound effect upon the gastric microvasculature of castrate males. Using intravascular fluorescent-albumin as a marker, ischaemia and leakage of fluid from capillaries into the extracellular compartment occurred, changes that are known to immediately precede the formation of gastric mucosal lesions.

It might therefore be predicted that HPA and sympathetic nervous system activation, following exposure to a range of stressors, would cause ischemia and subsequently promote the formation of gastric lesions (see Fig. 1).

Strategy I dasyurid males, which all appear to exhibit a high incidence of haemorrhage from stomach ulcers, and which are likely to experience psychosocial stress of olfactory origin, may provide very useful new animal models for future studies on the non-microbial pathogenesis of peptic ulcer in mammals.

CONTROL OF CORTICOSTEROID BINDING GLOBULIN CBG

In all Strategy I species that have been studied in some detail, castration causes an elevation in plasma CBG, an effect that is reversed by administration of testosterone (Bradley et al. 1980; McDonald et al. 1981). This inverse relationship between testosterone and CBG does not appear to exist in non-Strategy I species such as *Sminthopsis crassicaudata.* The absence of sex hormone binding globulin (SHBG) from the blood of all dasyurid marsupials (Bradley 1977; Sernia, Bradley and McDonald 1979; Bradley 1982b) would also appear to be important ensuring the exposure of tissues to high concentrations of androgen before and during the reproductive period.

A defect in glucocorticoid regulation

While the pituitary and hypothalamus are major sites involved in the regulation of GC feedback, the hippocampus plays a part by exerting an inhibitory effect on the HPA axis (Wilson 1985). This area of neuroendocrinology has received a great deal of attention, both in man and other mammals, since it was recognised that adrenocortical disruption may also be involved in ageing, depression and neurodegenerative diseases of man such as Alzheimer's disease.

It is now well known that in terminal semelparous dasyurid males *A. swainsonii* and *A. flavipes* (McDonald et al. 1986; Bradley 1990b) and in *A. subtropicus* (Bradley unpublished), a defect develops in glucocorticoid feedback. This defect seems to be an integral part of the physiological breakdown leading to the demise of males however in the absence of further information from studies on marsupials, one must look to studies on other vertebrates to explain why it might occur.

An extensive literature stretching back several decades has shown that prolonged stress or prolonged exposure to GCs, the adrenal steroids secreted during stress, can have adverse effects on the rodent hippocampus. Recent findings suggest a similar phenomenon in the human hippocampus associated with many neuropsychiatric disorders (Sapolsky 2000). GCs typify the double-edged quality of the stress-response. When secreted transiently during the brief physical challenge typical of mammalian stress, GCs aid survival. Glucocorticoids mobilise energy, increase cardiovascular tone, suppress non-essentials such as growth, tissue repair, and reproduction, and potentiate aspects of immunity while preventing activity to the point of autoimmunity (Munck et al. 1984; Sapolsky et al. 2001). However, excessive GCs can have deleterious consequences, including increased risks of hypertension, insulin-resistant diabetes mellitus, amenorrhea, impotency, ulcers, and immune suppression (Munck et al. 1984; Sapolsky et al. 2001). In addition, GCs can also have adverse effects on the nervous system, disrupting learning and memory, and synaptic plasticity (McEwen and Sapolsky 1995). Glucocorticoids can also have adverse morphologic effects, particularly in the hippocampus, a primary neural GC target site, with plentiful GC receptors. These effects include impairing neurogenesis, causing atrophy of dendritic processes and, sometimes, neurotoxic effects. The hippocampus has a variety of functions, but its best-documented function is in the realm of learning and memory. A vast literature shows that the structure plays a critical role in the consolidation of short-term into long-term explicit memory (Squire 1987).

GLUCOCORTICOIDS AND THE HIPPOCAMPUS

There is now considerable evidence that the hippocampus acts as a control site for the HPA axis (Jacobson and Sapolsky 1991; Van Eekelen and De Kloet 1992). Together with the amygdala and septal nuclei, the hippocampus is part of the limbic system that is concerned with olfaction and feeding behaviour (Goya et al. 1995). Furthermore the hippocampus, which is a primary target for GCs, is strongly implicated in the normal functions of mood, memory and learning. Continuous administration of GR antagonist improves cognitive function, while phasic blockade of brain GR function causes a cognitive deficit (Oitzl 1998). Capacity for learning new tasks may effectively be assessed in small mammals using the Morris water maze (Morris 1984) or Y mazes.

While it is accepted that hormones such as CRH or cortisol can be destructive when they are activated for long periods of time, or when the body is unable to terminate their production (Sapolsky 1992; McEwen 1998), another important advance in our knowledge of the stress response has been the introduction of the concept of *allostasis* (McEwen and Stellar 1993). The novelty of this concept of allostasis is that there may be no physiological set-point, with the possibility that any set-point is both fluid and changing (Sterling and Eyer 1981). The maintenance of life depends on the capacity of the organism to sustain its equilibrium via allostasis, in essence the ability to achieve stability through change.

One can envisage, within Strategy I males during the reproductive period, that the 'set-point' for free cortisol is considerably elevated, providing a short-term advantage, but in the longer-term leading to the demise of all males. The alteration of the set point may provide an adaptive advantage but accelerate GC mediated pathologies and senescence.

Sapolsky has significantly advanced our knowledge of the effects of glucocorticoids on the hippocampus (Sapolsky et al. 1984; Sapolsky 1985a, b; 1986a). A more subtle effect of GCs upon the

hippocampus may be atrophy of dendritic processes. This may compromise connectivity and function but not result in overt neuronal loss. Complexly arborised dendritic processes are prerequisites for the formation of elaborate neural networks, and GCs can atrophy such processes. Prolonged stress decreases numbers of apical dendritic branch points and the length of apical dendrites in hippocampal CA3 neurons in rodents and nonhuman primates (Sapolsky 1996; Regan and McEwen 1997). This effect is GC-dependent and can emerge after a few weeks of GC overexposure in rodents. Moreover, such atrophy correlates with impaired explicit memory. Thus, transient GC overexposure can alter neuronal morphologic features in a manner deleterious to explicit memory and with the abatement of the stressor or GC exposure, there is re-growth of dendritic processes. Psychosocial stress induced damage to the hippocampal dendritic tree, interneurons, neurogenesis or glia have been suggested (Lucassen et al. 2001) in the three shrew *Tupaia belangeri* where stress differentially affects apoptosis in hippocampal subregions.

Current studies in my laboratory on the brain of *A. subtropicus* employ confocal microscopy and selective staining of dendritic spines to assess the effect of various stressors on dendritic spine density. These changes are related to alterations in cognitive function using open field and radial-arm mazes, and the Morris water maze. Preliminary findings indicate that the short-term memory of *A. subtropicus* males is impaired late in their life in the wild and also with age in captivity. In contrast, the vomeronasal system appears to not undergo any morphological decline (Aland pers. comm.).

AGEING AND NEURODEGENERATIVE DISEASE

The hippocampus is known to be a focus of damage in several neurodegenerative conditions (Scully and Otten 1995). The hippocampus is the only site in the brain where a major loss of corticosteroid receptors has been observed (Angelucci et al. 1980; Sapolsky et al. 1983; Reul et al. 1988; De Kloet 1992). In the progressive age-related depletion, approximately half the corticosteroid receptors are lost. It is also clear that further significant changes occur with ageing while in some animal models, age related changes that are capable of influencing learning and memory, occur in senescent individuals.

The hippocampus is also a major target for damage in Alzheimer's disease in man (Pasquier et al. 1994). Elevated cortisol concentrations, increased urinary free cortisol excretion and defective HPA suppressibility have been reported in many patients with Alzheimer's disease (see review Seckl and Olsson 1995) and correlate with hippocampal damage (de Leon et al. 1988). In Alzheimer's disease, hippocampal GR and MR gene expression are maintained at control levels in surviving neurons, despite a generalised loss of neuronal gene expression and in the face of marked GC hypersecretion (Seckl et al. 1993). Elevated GC concentrations in Alzheimer's disease, perhaps as a consequence of neuronal loss in the hippocampus, might exert an even more potent deleterious effects via the maintained density of GC receptors in the remaining neurons (Goya et al. 1995). The attempt to maintain the hippocampal GR and MR at control levels may reflect a loss of plasticity following disruption of neurotransmitter inputs, since loss of afferent and efferent projections in Alzheimer's disease may isolate the hippocampus neuroanatomically (Hyman et al. 1984).

Preliminary studies (author in prep.) indicate that the hippocampus of *A. subtropicus* does possess saturable Type II glucocorticoid receptors, that the number of these receptors declines during the two weeks preceding the disappearance of males, and that morphological changes consistent with degeneration occur in the dentate gyrus and CA3 region of the hippocampus. It appears that beta-amyloid plaques and neurofibrillary tangles that are associated with neurodegenerative diseases in humans also occur in the brain of some Strategy I dasyurid males late in their life (for *A. stuartii* – McAllan and Norris pers. comm. and for *A. subtropicus* – Bradley unpublished). However, retrospective examination of brains from *A. stuartii* (*A. agilis*) shows that GC administration alone is insufficient to induce the formation of beta-amyloid plaque (Bradley and Masters unpublished).

A MARSUPIAL MODEL FOR STUDIES OF AGING AND SENESCENCE

Koolhaas et al. (1997) point out that our current understanding of the physiological mechanisms underlying stress related disorders is based not only on neuroendocrine and pharmacological studies in human patients but also on experimental studies in a wide variety of animal models. They indicate that while the contribution of social and physical environmental stress to the development of disease is of a general biological phenomenon in animals and man, one may criticise the validity of many of the current stress models. Koolhaas et al. (1997) comment that most of the animal models use stressors that bear little or no relationship to the biology of the species and suggest that more naturalistic models, that allow an experimental analysis of the aetiology and the temporal dynamics of the disease, should be used. Furthermore, in a review of the literature on stress and aging, Stein-Behrens and Sapolsky (1992) considered three model systems to be of particular significance for studies of stress, disease and mortality:

- programmed senescence in marsupial mice and fish as mediated by GC excess
- GC hypersecretion in rats and its role in damaging the aging brain, and
- potential human and primate adrenocortical dysfunction during ageing.

The fact that an adrenal hypersecretion results from ageing of the hippocampus through receptor depletion and/or neuron loss seems to provide an explanation for the inability of aged organisms to cope with stress. Although the HPA axis is manifestly altered with ageing, and the aged hippocampus is apparently involved in this altered response, the question remains: *Are GCs causally related to the process of ageing of the hippocampus?* The observations that chronic GC exposure is associated with neuronal loss in the hippocampus strongly implicate GCs in accelerating some aspects of aging (Goya et al. 1995). Consistent with this concept of hormonal acceleration of the ageing process Bradley (1997) argues that in another small dasyurid marsupial, *P. calura*, which shares the unusual truncated male life-history pattern, the strategy employed by males may be described by an *adaptive-stress senescence model.* This hypothesis draws upon the *senescence hypothesis* (Boonstra 1994) that represents a very significant advance in our understanding of factors controlling small mammal population in the northern hemisphere. In essence the *adaptive-stress senescence hypothesis* advocates an acceptance that for two individuals of the same biological age, one may be senescent as a consequence of experiencing a different hormonal *millieu* during a significant, albeit short part of its life-history. This clearly depends upon how ageing is defined and it is suggested that strict adherence to temporal-age paradigm is too restrictive, particularly when one considers species such as semelparous dasyurid marsupials with the associated hormonally distinct strategies which are employed by females and males.

This hypothesis suggests that stress related changes elevate plasma GCs having short-term beneficial effects upon intermediary metabolism to mobilise energy reserves to enable a heavy investment in a once only reproductive period, but in the long term the elevated GCs have deleterious effects, ultimately promoting pathogenic changes that finally lead to the death of males. While in the short term the physiological stress response is adaptive, in the longer term it leads to adverse changes that are consistent with senescence, in particular those effects that occur in the hippocampus affecting both morphology and function.

Although Barnett (1974) was unable to demonstrate accelerated ageing of male *Antechinus stuartii* relative to females of the same biological age using, as a criterion, the contraction of tail tendon collagen, it is postulated that post-breeding male *P. calura* are effectively senescent relative to females because of damage to critically sensitive brain areas. Males do show an impairment of their glucocorticoid feedback control (Bradley 1990b) and it is suggested that this involves damage to the hippocampal–hypothalamic–pituitary–adrenocortical axis as a consequence of exposure of hippocampal glucocorticoid receptors to high concentrations of free cortisol (Sapolsky 1996) during an adaptive elevation of the free cortisol concentration (Bradley 1987). A relationship between glucocorticoids and hippocampal damage has yet to be thoroughly investigated in marsupials.

Although the weight loss in male *P. calura* is not as profound as that which has been described in other semelparous dasyurid marsupial males, a thinning of the pelage does occur. Because this is accompanied, toward the end of the breeding season, by an increase in the aggressiveness of females toward males, and males which moved greater distance between captures at this time showed a greater incidence of rump and thigh fur loss and evidence of bite marks, it is reasonable to postulate that, at least for *P. calura* and possibly for other species, the female aggression and the male dispersal are causally related. Bearing in mind that these events occur at a time of year when the nights are quite cold, and the males which move long distances are generally trapped at some distance from potential nest trees, it is also reasonable to postulate that these males are exposed to a considerable thermoregulatory challenge.

A consistent observation with *P. calura,* and indeed with all other semelparous dasyurid marsupial males (pers. obs.), is that when individuals are removed from traps in a hypothermic state, they will invariably die within 24 hours in spite of all measures that are taken and the proximate cause of death can usually be identified as acute haemorrhage from gastric ulcers. Similar evidence of bleeding from GI ulcers occuring up to 20 days before the death of male *P. tapoatafa* was reported by Soderquist and Ealey (1994). Furthermore post-mortem investigation of four freshly dead male *P. tapoatafa* also revealed that while ectoparasites were not apparent in the GI tract, lungs or heart, all showed evidence of peptic or intestinal ulceration.

Behavioural changes

In some dasyurid species a change in behaviour between the sexes during the life-history can result in an increase in plasma cortisol in the male. In *Phascogale calura* before and during the mating period the males are clearly dominant in their pursuit of females, however when females are pregnant they become very aggressive toward males (Bradley 1997). In another semelparous species, *Phascogale taqpoatafa,* Soderquist and Ealey (1994) has also reported an increase in female aggression toward males during pregnancy. This is considered to be a potent stimulus for the post-mating terminal dispersal of males (Soderquist and Ealey 1994; Bradley 1995, 1997).

Braithwaite (1974, 1979) reported that behavioural changes occur during the life-history of *A. stuartii* (*A. agilis*). Further behavioural studies on *A. stuartii* (Scott 1987) reported that agonistic encounters between caged males caused in an elevation of plasma cortisol, but that the experience of mating could result in a lowering of plasma cortisol. Lazenby-Cohen and Cockburn (1991) suggested that after a resolution of agonistic interactions early during the life-history, males would cohabit with other males during mating.

While contrived encounters between caged individuals have been criticised and may not give results that can reliably be used

to determine the nature of interactions in the field, the difficulty in observing natural interactions explain a general deficit in our knowledge in this area. Further studies that relate social and physiological changes are clearly warranted.

Starvation and negative nitrogen balance

It is curious that at a time when their plasma free cortisol is elevated, *A. stuartii* males do not appear to seek and ingest food during the last days before their disappearance. This may be deduced from a common finding that the stomachs of males are empty. However, stress has been reported to suppress feeding even in food deprived animals (Krahn et al. 1986), an effect that may be mediated by CRH, a potent anorexic agent (Sapolsky et al. 2001). CRH agonists are known to block the anorexic effect of stress (Arase et al. 1988). If hippocampal inhibition of CRH release is diminished in Strategy I dasyurid males CRH release may be sufficient to exert this type of anorexic effect and explain the decreased food intake. During the mating period males undergo a significant reduction in body mass that is consistent with a state of negative nitrogen balance. However the apparent decrease in body mass may be disguised by a glucocorticoid induced increase in total body water TBW (Nagy et al. 1978).

It is reasonable to predict that the survival time for a starving animal should be related to body size and condition at the commencement of fasting. At the commencement of the reproductive period males are in good condition. Because of surface to volume considerations there will be a relationship between body size and development of hypothermia during nitrogen mobilisation/negative nitrogen balance. The threshold would be affected by predictable adverse climatic conditions during the terminal phase and also by altitude and latitude.

It is thought that Strategy I males during the mating period, spend little time searching for food sources, instead devoting their energy to locating females and mating. There is a clear sexual dimorphism in these species, with males being considerably larger than females at the beginning of the mating period. Males therefore have some potential energy reserves but to fully exploit these, males would need to starve themselves. In *A. stuartii* during late winter it is difficult for males to procure food and engage in mating because mating takes place near communal aggregations that are often a considerable distance from male foraging areas (Lazenby-Cohen and Cockburn 1988, 1991; Cockburn and Lazenby-Cohen 1992; Cockburn 1997). There is also evidence that the males enter negative nitrogen balance (Woollard 1971) during which proteins are presumably being utilised for energy sources after carbohydrate and fat reserves have been expended. *A. stuartii* (*agilis*) males exhibit haemoglobinuria, ketonuria, proteinuria, glycosuria, particularly during the terminal two weeks (Bradley 1977). Consistent with a disturbance of intermediary metabolism associated with starvation is the observation that some male *A. stuartii* (*A. agilis*) in this terminal phase show evidence glycosuria, haemoglobinuria , proteinuria, and sometimes, ketonuria (Bradley 1977). The finding of ketonuria is consistent with the metabolic alterations that occur during starvation while the proteinurias and haemoglobinurias are consistent with the renal glomerular damage that has been reported by McAllan et al. (1996) and which may be reproduced in the laboratory by exogenously administering a combination of testosterone and cortisol (McAllan et al. 1997, 1998). A finding of glomerulonephritis and tubular necrosis, affecting in particular the proximal convoluted tubule, is common in male *A. subtropicus* during the last days before their disappearance from the population (author in prep.)

To explain the life-history of semelparous dasyurid species in general, and of *P. calura* in particular, Bradley (1997) has proposed the '*adaptive stress senescence hypothesis*' to explain that males become prematurely senescent as a consequence of exposure to high concentrations of free cortisol for a protracted period prior to their disappearance from the population. The critical factors that seem to predispose males to the development of pathological changes are outlined in Table 1.

Periodic semelparity or plasticity of life-history

Some dasyurid species exhibit a high or even complete male mortality during some years while in other years many males may survive beyond the breeding period and appear to be capable of breeding in the next breeding period. While *Dasyurus hallucatus* may show an incomplete male mortality in some years (Schmitt et al. 1981) they disappear in others (Dickman and Braithwaite 1992) or the phenomenon may occur annually (Oakwood et al. 2001). *D. hallucatus* would appear to be the largest dasyurid species in which a complete male mortality may sometimes occur.

Periodic semelparity may also occur in *Parantechinus apicalis*. While males of this species have been reported to undergo the 'male die-off' (Dickman and Braithwaite 1992), males in the island population may sometimes survive to breed in a second year (Woolley 1991), with survival of males in an island population being recorded in three successive years from 1997 (Mills and Bencini 1999, 2000).

A moribund male *Parantechinus apicalis* found on Boullanger Island toward the end of the breeding period (Bradley and Dickman unpubl. obs.) was found to have acute haemorrhagic ulceration of the gastric mucosa, with a blood sample revealing elevated testosterone (1.49 µg/dl), high cortisol (6.58 µg/dl), low corticosteroid binding globulin (CBG) (0.92 µg/dl) and low albumin.(1.14 g/dl). The free cortisol concentration would be high and this is consistent with the endocrine profile of Strategy I males in their terminal phase.

Curiously the male *D. hallucatus* in the study of Oakwood et al. (2001) did not show cortisol concentrations that were significantly higher than those of the females during the reproductive period. However a significantly higher free cortisol concentration in males could have resulted from decreases in both CBG

Table I Critical factors that appear to be significant in allowing Strategy I dasyurid marsupial males to develop their unique adaptive physiological response.

- The absence of sex-hormone–binding globulin in all dasyurid marsupials
- Maintenance of luteinising hormone (LH) production and testosterone release from Leydig cells (→ *consistent with a maintenance of gonadotrophin-releasing hormone release*)
- Failure of spermatogenesis *circa* two months before reproductive period and maintenance of elevated testosterone concentration (→ *consistent with a defect in follicle stimulating hormone (FSH) release and/or target insensitivity*)
- The inverse relationship between plasma testosterone and plasma CBG (→ *rises in plasma testosterone cause decrease in plasma CBG*)
- Androgen-dependent increase in activity of scent glands coinciding with increase in activity of males (→ *stimulates increase in apparent density*)
- A terminal suppression of appetite (→ *reduction in food intake*)
- Major adjustments and sequential change in intermediary metabolism of carbohydrates, fats and proteins in Strategy I males (→ *consistent with mobilisation of energy reserves and starvation*)
- A terminal decrease in plasma albumin in Strategy I males (→ *effect upon steroid partitioning to deliver more free cortisol*)
- Damage to hippocampal neurons (→ *GC receptor decrease and reduction in dendritic connectivity*)
- The development of a defect in glucocorticoid feedback (→ *high concentrations of free cortisol are maintained*)
- Olfactory stimulation of stress pathways also involving hippocampus (→ *activate HPA axis, stimulate increase in free cortisol and cause damage to hippocampal neuron GC receptors and dendritic connectivity*)
- Terminal decline in olfactory function in males with reliance upon vomeronasal system to locate females
- Concurrent terminal deficit in short term-memory and spatial orientation in males.
- Increase in aggressiveness of pregnant females toward males (→ *stimulates terminal dispersal of males*)
- •Vagrant males lacking insulation (both fats and pelage thinning) (→ *more susceptible to hypothermia in cold conditions*)
- Sympathetic stimulation combining with CRH and GCs to cause gastric mucosal ischemia, increased capillary permeability and inhibit repair mechanisms (→ *leads to development of mucosal erosions and acute haemorrhage from ulcers*)
- Androgens and free GC elevated causing renal glomerular damage (→ *proteinuria and haemoglobinuria contributing to anaemia and general debilitated state*)

and albumin concentrations. Such changes, generating an elevated free cortisol concentration and associated immune suppression, would be consistent with the observed weight loss, parasite infections and anaemia. *D. hallucatus*, because it appears to differ in the terminal physiological and pathological changes, clearly warrants further investigation.

Plasticity of life-history strategy in several dasyurid species presents a most interesting prospect. If a failure of glucocorticoid regulation is involved in the male mortality, as current evidence suggests, it might be predicted that in years in which males survive the reproductive period:

- Density would be lower (implications for olfactory communication and consequent stress).
- Plasma testosterone would not be elevated to the same degree as in years when all males disappear.
- Plasma CBG would be higher (CBG depression varies between years in males).
- Plasma free cortisol would be lower.
- Less damage would be caused to hippocampal glucocorticoid receptors and a glucocorticoid feedback defect would not develop.
- Stress-related white blood cell changes would not be found (indicative of general maintenance of immune competence).
- Lesions would not develop in gastric mucosa and renal glomeruli.

At high density male mortality may be more significant than when the population is at low density from which many males may survive the reproductive period. Evidence for these physiological observations would be consistent with the operation of a density dependent population regulation, but it is suggested that density should be regarded in a *behavioural/olfactory* context that would be exacerbated by any reproductive related social aggregation such as occurs during the reproductive period. For example individuals moving more extensively, and using chemical communication, would be expected have a considerable influence on conspecifics (see Fig. 1). It is also important to determine whether males that survive the reproductive period are fertile during the next reproductive-period or whether spermatogenesis ceases during the first year without resumption as in 'obligate' Strategy I dasyurid males.

Single males surviving the reproductive period?

Very occasionally, in populations in which males are regarded as exhibiting a Strategy I life-history, single 'males' may be trapped after the majority of males have disappeared. During extensive trapping for *A. swainsonii* and *A. stuartii* (*A. agilis*), *A. flavipes*, and *A. stuartii* (*A. subtropicus*) the author has captured 2, 1, 2 and 1 respectively of these 'males'. In both cases for the *A. swainsonii* the scrotum either had not formed or had been lost. The absence of a scrotum was also noted in the other cases however one *A. flavipes* had a scrotum but the testes were very small and flaccid. In all cases the plasma testosterone concentration was either very low or undetectable. The absence of a scrotum and testes would explain the low plasma testosterone. For the two *A. swainsonii* the plasma CBG concentration was 9.3 and 10.7 µg/dl while for the two *A. flavipes* the values were 8.2 and 9.9 µg/dl. These blood samples were collected from males approximately two months after the disappearance of all other males and the high CBG values are consistent with the low plasma testosterone concentrations. In magnitude these CBG concentrations resemble those found in pregnant females and these elevated CBG concentrations would provide considerable buffering potential for cortisol, resulting in the exposure of

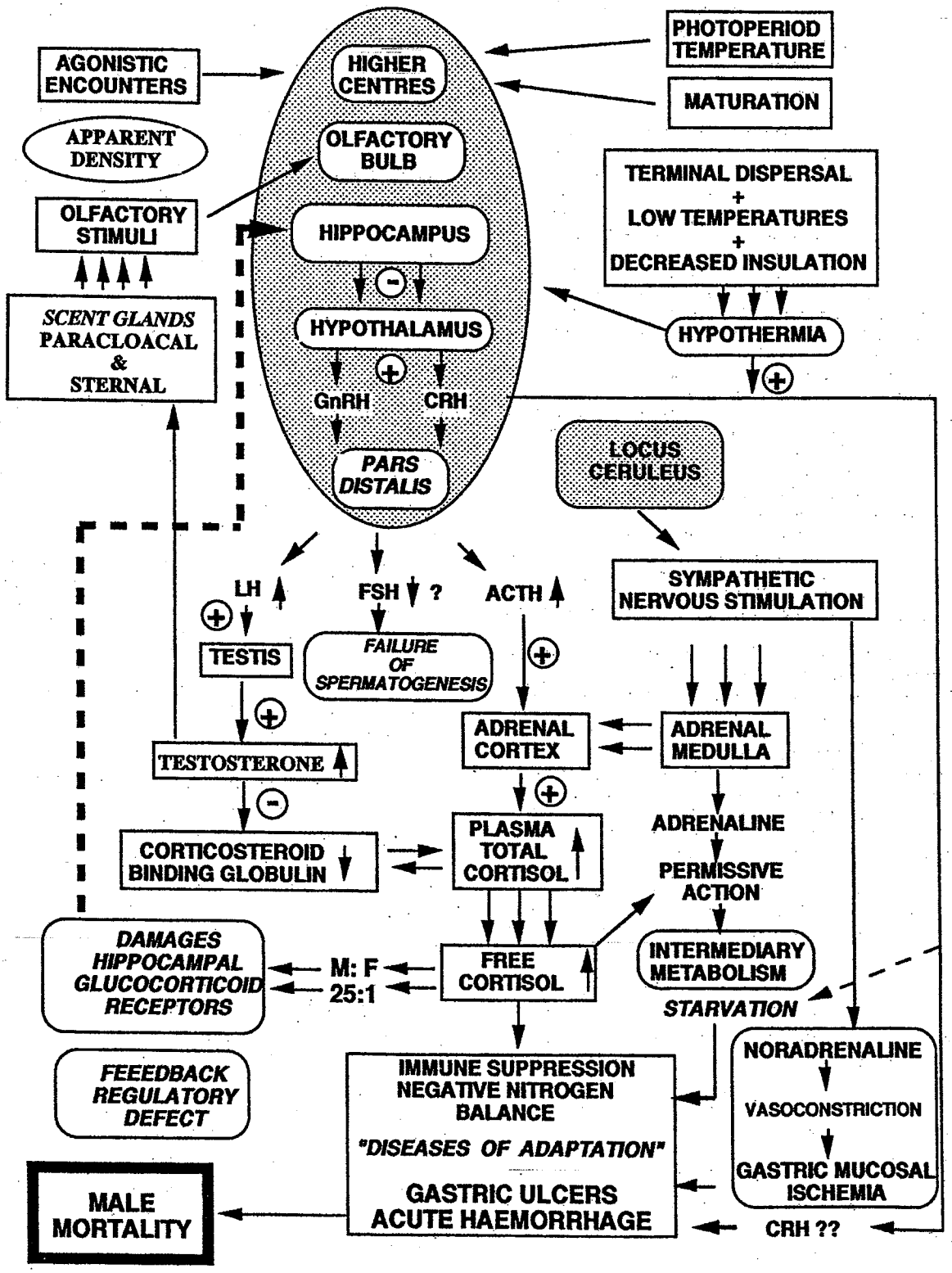

Figure 1 Diagram showing the likely relationships between environmental and physiological factors that may explain why males die at the end of the reproductive period (modified after Bradley 1978; 1985). CNS indicated by stippled areas.

tissues to relatively low free cortisol concentrations even during environmentally stressful periods involving HPA stimulation.

Since cutaneous scent glands in marsupials appear to be controlled by androgens, the absence of testes could indicate that the scent producing glands would be non-functional. This could convey to other males and females the signal that these 'males' were not competitors (Bradley and Monamy 1991). The absence of the characteristic semiochemical from males could explain the persistence of these odd 'males' during and beyond the reproductive period. In *A. flavipes* and *A. stuartii* (*A. subtropicus*) males castrated four months before the breeding season and released may be recaptured beyond the time when all other males have disappeared (Bradley unpubl. obs.). These observations are consistent with an important signalling role for scent glands in males. The survival of these odd 'males' is considered to be independent of the operation of a plasticity of life-history that might explain the persistence of a significant number of adult males in some years.

Why do females survive beyond the breeding period?

The physiological profile of females differs markedly from that of males, particularly during the breeding period and beyond when all females are pregnant. When males undergo an androgen dependent decrease in plasma CBG concentration, the plasma CBG concentration in females gradually increases. This may be attributable to the action of female reproductive hormones, particularly oestradiol. As a consequence of this increase in CBG in females, they are in effect insulated from the effects of an elevation in cortisol – that is they are more *stress resistant* at this time during pituitary-adrenocortical stimulation. This would explain why males in their terminal phase, but not females, exhibit negative nitrogen balance (Woollard 1971; Bradley unpublished). Furthermore, females do appear to consume food when males are reported to reduce their food consumption.

While the abovementioned studies attribute the male mortality to a variety of causes, based upon my experiences working with small dasyurid marsupials for over 25 years I am convinced that the proximate cause of death in the majority of cases is a consequence of haemorrhage from stress induced gastric ulcers. This view is based upon the results of necropsies carried out on many individuals covering several small Strategy I dasyurid marsupial species during this time. Stress induced immune suppression and the development of various pathologies affecting the renal and hepatic systems would also appear to contribute significantly to the generally debilitated physiological state of males leading ultimately to their death.

Future directions

This chapter includes some material that is at present speculative and deserves further investigation. It is included for the specific purpose of providing new directions in which research might proceed based upon exciting studies in other mammals. It is suggested that Strategy I dasyurid marsupials, because of the unique life-history strategy and associated physiological control, present us with a unique opportunity to address fundamental neuroendocrine questions that may be of relevance to all mammals.

Koolhaas et al. (1997) perceptively point out that most animal models use stressors that bear little or no relationship to the biology of the species (to the situations that an animal may meet in its everyday life in a natural habitat). They advocate the use of more naturalistic animal models that allow an experimental analysis. Strategy I dasyurid marsupials, because of their unique biological attributes, present us with exciting opportunities for future research. We would be wise to follow this advice.

References

Angelucci, L., Valeri, P., & Grossi, E. (1980), 'Involvement of hippocampal corticosterone receptors in behavioral phenomena', in *Progress in Psychoneuroendocrinology* (eds. G. Brambilla, G. Rascagni, & D. de Wiede), Elsevier, Amsterdam.

Arase, K., York, D., Shimizu, H., Shargill, N., & Bray, G. (1988), 'Effects of corticotropin-releasing factor on food intake and brown adipose tissue thermogenesis in rats', *Am J Physiol*, **255**:E255–9.

Barker, I.K., Beveridge, I., Bradley, A.J., & Lee, A.K. (1978), 'Observations on spontaneous stress related mortality among males of the dasyurid marsupial *Antechinus stuartii* (Macleay)', *Aust J Zool*, **26**:435–47.

Barker I.K., Carbonell P.L., & Bradley A.J (1981), 'Cytomegalovirus infection of the prostate in the dasyurid marsupials *Phascogale tapoatafa* and *Antechinus stuartii*', *J Wildl Diseases*, **17**:433–41.

Barnett, J.L. (1973), 'A stress response in *Antechinus stuartii* (Macleay)', *Aust J Zool*, **21**:501–13.

Barnett, J.L. (1974), 'Changes in hydroxyproline concentration of the skin of *Antechinus stuartii* with age and hormonal treatment', *Aust J Zool*, **22**:311–18.

Boonstra, R. (1994), 'Population cycles in microtenes: the senescence hypothesis', *Evol Ecol*, **8**:196–219.

Bondy, P. (1981), 'Disorders of the adrenal cortex', in *Williams Textbook of Endocrinology, 7th ed.* (eds. J. Wilson, & D. Foster), Saunders, Philadelphia.

Bradley, A.J. (1977), 'Stress and mortality in *Antechinus stuartii* (Macleay)', PhD thesis, Monash University, Melbourne, Australia.

Bradley, A.J. (1982a), 'The biology of the Red-tailed Phascogale, *Phascogale calura*', *Arid Zone Newsletter*:16–18.

Bradley, A.J. (1982b), 'Steroid binding proteins in the plasma of dasyurid marsupials', in *Carnivorous Marsupial* (ed. M. Archer): 651–7, Surrey Beatty & Sons, Sydney.

Bradley, A.J. (1985), 'Steroid binding proteins and life history in marsupials', in *Current Trends in Comparative Endocrinology Vol. 1* (eds. B. Lofts, & W.N. Holmes): 269–70, Hong Kong University Press, Hong Kong.

Bradley, A.J. (1987), 'Stress and mortality in the Red-tailed Phascogale *Phascogale calura* (Marsupialia: Dasyuridae)', *Gen Comp Endocr*, **67**:85–100.

Bradley, A.J. (1990a), 'Seasonal effects on the haematology and blood chemistry in the Red-tailed Phascogale, *Phascogale calura* (Marsupialia: Dasyuridae)', *Aust J Zool*, **37**:533–43.

Bradley, A.J. (1990b), 'Failure of glucocorticoid feedback during breeding in the male Red-tailed Phascogale *Phascogale calura* (Marsupialia: Dasyuridae)', *J Steroid Biochem Molec Biol*, **37**:155–63.

Bradley, A.J. (1995), 'Red-tailed Phascogale *Phascogale calura*', in *Mammals of Australia* (ed. R. Strahan): 102–3, Reed Books, Sydney.

Bradley, A.J. (1997), 'Reproduction and life-history in the red-tailed phascogale, *Phascogale calura* (Marsupialia: Dasyuridae): The adaptive–stress senescence hypothesis', *J Zool Lond*, **241**:739–55.

Bradley, A.J. & Monamy, V.A. (1991), 'A physiological profile of *Antechinus swainsonii* (Marsupialia: Dasyuridae) males surviving the post-mating mortality', *Aust Mammal*, **14**:25–7.

Bradley, A.J., McDonald, I.R., & Lee, A.K. (1975), 'Effect of exogenous cortisol on mortality of a dasyurid marsupial', *J Endocrinol*, **66**:281–2.

Bradley, A.J., McDonald, I.R., & Lee, A.K. (1976), 'Corticosteroid binding globulin and mortality in a dasyurid marsupial', *J Endocrinol*, **70**:323–4.

Bradley, A.J., McDonald, I.R., & Lee, A.K. (1980), 'Stress and mortality in a small marsupial (*Antechinus stuartii* Macleay)', *Gen Comp Endocr*, **40**:188–200.

Braithwaite, R.W. (1974), 'Behavioural changes associated with the population cycle of *Antechinus stuartii* (Marsupialia)', *Aust J Zool*, **22**:45–62.

Braithwaite, R.W. (1979), 'Social dominance and habitat utilization in *Antechinus stuartii* (Marsupialia)', *Aust J Zool*, **27**:517–28.

Braithwaite, R.W., & Lee, A.K. (1979), 'A mammalian example of semelparity', *Am Nat*, **113**:151–5.

Capani de Kaski, M., Rentsch, R., Levi, S., & Hodgson, H.J. (1995), 'Corticosteroids reduce regenerative repair of epithelium in experimental gastric ulcers', *Gut*, **37**:613–16.

Cheal, P.D., Lee, A.K., & Barnett, J.L. (1976), 'Changes in the haematology of *Antechinus stuartii* (Marsupialia), and their association with male mortality', *Aust J Zool*, **24**:299–311.

Cockburn, A. (1989), 'Adaptive patterns in marsupial reproduction', *Trends in Ecology and Environment*, **4**:126–31.

Cockburn, A. (1992), 'Evoltionary ecology of the immune system: Why does the thymus involute?', *Functional Ecology*, **6**:364–70.

Cockburn, A. (1997), 'Living slow and dying young: senescence in marsupials', in *Marsupial Biology: Recent Research, New Perspectives* (ed. N.R. Saunders, & L.A. Hinds), pp. 163–71, Sydney, University of New South Wales Press.

Cockburn, A., & Lazenby-Cohen, K.A. (1992), 'Use of nest trees by *Antechinus stuartii*, a semelparous lekking marsupial', *J Zoology Lond* **226**:657–80.

Cuttle, P. (1978), 'The behaviour in captivity of the dasyurid marsupial *Phascogale tapoatafa* (Meyer)', MSc thesis, Monash University, Melbourne, Australia.

Cuttle, P. (1982), 'Life history strategy of the dasyurid marsupial, *Phascogale tapoatafa*', in *Carnivorous Marsupials* (ed. M. Archer), pp. 13–22, Sydney, Roy. Zool. Soc. NSW.

De Kloet, E.R. (1992), 'Corticosteroids, stress and aging', *Ann NY Acad Sci*, **663**:357–71.

Dickman, C.R., Parnaby, H.E., Crowther, M.S., & King, D.H. (1998), '*Antechinus agilis* (Marsupialia: Dasyuridae), a new species from the *A. stuartii* complex in south-eastern Australia', *Australian Journal of Zoology*, **46**:1–26

Dickman, C.R., & Braithwaite, R.W. (1992), 'Post-mating mortality mortality of males in the dasyurid marsupials, *Dasyurus* & *Parantechinus*', *J Mammal*, **73**:143–7.

Filaretova, L.P., Filaretova, A.A., & Makara, G.B. (1998), 'Corticosterone increase inhibits stress-induced gastric erosions in rats', *Am J Physiol*, **274**:G1024–30.

Filaretova, L.P., Maltcev, N., Bogdanov, A., & Levkovich, Y. (1999), 'Role of gastric microcirculation in the gastroprotection by glucocorticoids released during water-restraint stress in rats', *Chin J Physiol*, **42**:145–52.

Goya, L., Rivero F., & Pascual-Leone, A.M. (1995), 'Glucocorticoids, stress and aging', in *Hormones and Aging* (eds. P.S. Timiras, W.D. Quay, & A. Vernadakis), pp. 249–64, CRC Press, New York.

Hamerton, A.E. (1934), 'Report on deaths occurring in the societys gardens during the year 1933', *Proc Zool Soc Lond*, **104**:389–422.

Hamerton, A.E. (1935), 'Report on deaths occurring in the societys gardens during the year 1934', *Proc Zool Soc Lond*, **105**:433–74.

Hamerton, A.E. (1938), 'Report on deaths occurring in the societys gardens during the year 1937', *Proc Zool Soc Lond*, **108**:489–526.

Hamerton, A.E. (1939). 'Review of mortality rates and report on the deaths occurring in the society's gardens during the year 1938', *Proc Zool Soc Lond*, **108**:281–27.

Hanlon, A.J. (2001), 'The pathogenesis of gastric ulcer in a small marsupial mouse (*Antechinus subrtopicus*)', BSc Hons thesis, University of Queensland.

Harding, R.K., & Morris, G.P. (1977), 'Cell loss from normal and stressed gastric mucosae of the rat', *Gastroenterology*, **72**:859–63.

Hernandez, D.E. (1990), 'The role of brain peptides in the pathogenesis of experimental stress gastric ulcers', *Ann NY Acad Sci*, **597**:28–35.

Hosoda, S., Ikedo, H., & Saito, T. (1981), '*Praemys (Mastomys) natalensis*: Animal model for study of histamine–induced duodenal ulcers', *Gastroenterology*, **80**:16–21.

Hyman, B.T., van Hoesen, G.W., Damasio, A.R., & Barnes, C.L. (1984), 'Alzheimer's disease: cell-specific pathology isolates the hippocampal formation', *Science*, **225**:1168–70.

Jacobson, J.A., & Sapolsky, R.M. (1991), 'The role of the hippocampus in feedback regulation of the hypothalamic–pituitry–adrenocortical axis', *Endocrne Rev*, **12**:118–34.

Kawano, S., Sato, N., Tsuji, S., Hayashi, N., Tsuiji, M., Masuda, E., Takei, Y., Nagano, K., Fusamoto, H., Ogihara, T., Miwa, H., Hamada, T., & Kamada, T. (1991), 'Two dimensional computer color graphics of gastric mucosal blood distribution in normal subjects and ulcer patients', *Endoscopy*, **23**:317–20.

Kitagawa, H., Fujiwara, M., & Osumi, Y. (1979), 'Effects of water immersion stress on gastric secretion and mucosal blood flow in rats', *Gastroenterology*, **77**:298–302.

Kitchener, D.J. (1981), 'Breeding, diet and habitat preference of *Phascogale calura* (Gould, 1844) (Marsupialia: Dasyuridae) in the southern wheat belt, Western Australia', *Rec West Aust Mus*, (Suppl.) **9**:173–86.

Kivilaakso, E., Barzilai, A., Schiessel, R., Fromm, D., & Silen, W. (1981), 'Experimental ulceration of rabbit antral mucosa', *Gastroenterology*, **80**:77–83.

Koolhaas, J.M., Meerlo, P., De Boer, S.F., Strubbe, J.H., & Bohus, B. (1997), 'The temporal dynamics of the stress response', *Neuroscience and Behavioral Reviews*, **21**:775–82.

Krahn, D., Gosnell, B., Grace, M., & Levine, A. (1986), 'CRF antagonist partially reverses CRF- and stress-induced effects on feeding', *Brain Res Bull*, **17**:285–9.

Krajewski, C., Woolley, P.A., & Westerman, M. (2000), 'The evolution of reproductive strategies in dasyurid marsupials: implications of molecular phylogeny', *Biol J Linn Soc*, **71**:417–35.

Krakoff, L. (1988), 'Glucocorticoid excess syndromes causing hypertension', *Cardiology Clinic*, **6**:537–45.

Kuwayama, H., Matsuo, Y., & Eastwood, G.L. (1989), 'Effects of hydrocortisone sodium succinate on the healing of established gastric ulcers in the rat', *Dig Dis Sci*, **34**:A7.

Lazenby-Cohen, K.A., & Cockburn, A. (1988), 'Lek promiscuity in a semelparous mammal, *Antechinus stuartii* (Marsupialia: Dasyuridae)', *Behavioral Ecology and Sociobiology*, **22**:195–202.

Lazenby-Cohen, K.A., & Cockburn, A. (1991), 'Social and foraging components of the home range in *Antechinus stuartii* (Marsupialia: Dasyuridae), *Aust J Ecol*, **16**:301–7.

Lee, A.K., Bradley, A.J., & Braithwaite, F.W. (1977), 'Corticosteroid levels and male mortality in *Antechinus stuartii*', in *Biology and Environment* (eds. B. Stonehouse & D. Gilmore), pp. 209–20, Macmillan, London.

Lee, A.K., & Cockburn, A. (1985a), *The evolutionary ecology of marsupials*, Cambridge University Press, Cambridge.

Lee, A.K., & Cockburn, A. (1985b), 'Spring declines in small mammal populations', *Acta Zool Fenn*, **173**:75–6.

Lee, A.K., & McDonald, I.R. (1985), 'Stress and population regulation in small mammals', in *Oxford reviews of reproductive biology*, pp. 261–304, Oxford University Press, Oxford.

Lee, A.K., Woolley, P., & Braithwaite, R.W. (1982), 'Life history strategies of dasyurid marsupials', in *Carnivorous Marsupials* (ed. M. Archer), pp. 1–11, Royal Society of New South Wales, Sydney, Australia.

de Leon, M.J., McRae, T., Tsai, J.R., George, A.E., Marcus, D.L., Freedman, M., Wolf, A.P., & McEwen, B.S. (1988), 'Abnormal cortisol response in Alzheimer's disease linked to hippocampal atrophy', *Lancet*, **ii**:391–2.

Lorini, M.L., Deoliveira, J.A., & Persson, V.G. (1994), 'Annual age structure and reproductive patterns in marmosa incana (Lund, 1841) (Didelphidae, Marsupialia)', International *J Mammal Biol*, **59**:65–73.

Lucasen, P.J., Vollmann-Honsdorf, G.K., Gleisberg, M., Boldizsar-Czeh, E., De Kloet, R., & Fuchs, E. (2001), 'Chronic psychosocial stress diffrerentially affects apoptosis in hippocampal subregions and cortex of the adult tree shrew', *Eur J Neurosci*, **14**:161–6.

Martin, M., & Menguy, R. (1970), 'Influence of adrenocorticotropin, cortisone, asprin, and phenylbutazone on the rate of exfoliation and the rate of renewal of gastric mucosal cells', *Gastroenterology*, **58**:329–36.

Marshall, B.J., McCallum, R.W., & Guerrant, R.L. (1991), *Helicobacter pylori in peptic ulceration and gastritis*, Boston. Blackwell.

McAllan, B., Roberts, J.B., & O'Shea, T. (1996), 'Seasonal changes in renal morphometry of *Antechinus stuartii* (Marsupialia: Dasyuridae)', *Aust J Zool*, **44**:337–54.

McAllan, B., Roberts, J.B., & O'Shea, T. (1997), 'Effects of testosterone and cortisol on the renal morphology of male *Antechinus stuartii* (Marsupialia)', *Gen Comp* Endocr, **107**:439–49.

McAllan, B., Roberts, J.B., & O'Shea, T. (1998), 'The effects of cortisol and testosterone on renal function in male *Antechinus stuartii* (Marsupialia)', *J Comp Physiol B*, **168**:248–56.

McDonald, I.R., Lee, A.K., Bradley, A.J., & Than, K.A. (1981), 'Endocrine changes in dasyurid marsupials with differing mortality patterns', *Gen Comp Endocrinol*, **44**:292–301.

McDonald I.R., Lee A.K., Than K.A., & Martin R.W.(1986), 'Failure of glucocorticoid feedback in males of a population of small marsupials (*Antechinus swainsonii*) during the period of mating', *J Endocrinol*, **108**:63–8.

McEwen, B.S. (1998), 'Stress, adaptation, and disease. Allostasis and allostatic load', *Ann NY Acad Sci*, **840**:33–44.

McEwen, B. (1999), 'Stress and hippocampal plasticity', *Ann Rev Neurosci*, **22**:105–22.

McEwen, B.S., DeKloet, E.R., & Rostene, W. (1986), 'Adrenal steroid receptors and actions in the nervous system', *Physiol Rev*, **66**:1121–88.

McEwen, B., & Sapolsky, R. (1995), 'Stress and cognitive function', *Curr Opin Neurobiol*, **5**:205–11.

McEwen, B.S., & Stellar, E. (1993), 'Stress and the individual. Mechanisms leading to disease', *Arch Intern* Med, **27**:2093–101.

Mills, H., & Bencini, R. (1999), 'Postmating survival of male dibblers *(Parantechinus apicalis)* in island populations', *Australian Mammal Society Newsletter*, Nov, p. 19.

Mills, H.R., & Bencini, R. (2000), 'New evidence for facultative male die-off in island populations of dibblers, *Parantechinus apicalis*', *Aust J Zool*, **48**:501–10.

Moore, G.H. (1974), 'Aetiology of the die-off of male *Antechinus stuartii*', PhD thesis, Australian National University, Canberra, Australia.

Morris, R.G.M. (1984), 'Developments of a water-maze procedure for studying spatial learning in the rat', *J Neurosci Methods*, **11**:47–60.

Munch, A., Guyre, P., & Holbrook, N. (1984), 'Physiological functions of glucocorticoids in stress and their relation to pharmacological actions', *Endocr Rev*, **5**:25–42.

Murphy, H.M., Wideman, C.H., & Brown, T.S. (1979), 'Plasma corticosterone levels and ulcer formation in rats with hippocampal lesions', *Neuroendocrinol*, **28**:123–30.

Nagy, K.A., Seymour, R.S., Lee, A.K., & Braithwaite, R. (1978), 'Energy use and water budgets in free living *Antechinus stuartii* (Marsupialia: Dasyuridae)', *J Mammalogy*, **59**:60–8.

Oakwood, M. (1999), 'Reproduction and demography of the Northern quoll, *Dasyurus hallucatus*, in the wet–dry tropics of Northern Australia', *Australian Mammal Society Newsletter*, Nov. p. 15.

Oakwood, M., Bradley, A.J., & Cockburn, A. (2001), 'Semelparity in a large marsupial', *Proc R Soc Lond B*, **268**:407–11.

Oitzl, M.S., Fluttert, M., Sutanto, W., & de-Kloet, E.R. (1998), 'Continuous blockade of brain glucocorticoid receptors facilitates spatial learning and memory in rats', *European J Neurosci*, **10**:3759–66.

Pasquier, F., Bail, L., Lebert, F., Pruvo, J.P., & Petit, H. (1994), 'Determination of medial temporal lobe atrophy in early Alzheimer's disease with computed tomography', *Lancet*, **343**:861–2.

Pine, R. (1994), 'Sex and death', *Aust Nat Hist*, **24**:4.

Regan, L.P., & McEwen, B.S. (1997), 'Controversies surrounding glucocorticoid-mediated cell death in the hippocampus', *J Chem Neuroanat*, **13**:149–67.

Reul, J.M., Tonnaer, J.A., & Kloet, E.R. (1988), 'Neurotropic ACTH analogue promotes plasticity of type I corticosteroid receptor in brain of senescent male rats', *Neurobiol Aging*, **9**:253–60.

Sapolsky, R.M. (1985a), 'A mechanism for glucocorticoid toxicity in the hippocampus increased neuronal vulnerability to metabolic insults', *J Neurosci*, **5**:1228–32.

Sapolsky, R.M. (1985b), 'Glucocorticoid toxicity in the hippocampus: temporal aspects of neuronal vulnerability', *Brain Res*, **359**:300–5.

Sapolsky, R.M. (1986), 'Glucocorticoid toxicity in the hippocampus: reversal by supplementation with brain fuels', *J Neurosci*, **2**:2240–7.

Sapolsky, R.M. (1992), 'Neuroendocrinology of the stress response' in *Behavioural Endocrinology* (eds. J.B. Becker, S.M. Breedlove, & D. Grews), Chp 10, MIT Press, Massachusetts, USA, 287 p.

Sapolsky, R.M. (1996), 'Why stress is bad for your brain', *Science*, **273**:749–50.

Sapolsky, R.M. (2000), 'Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders', *Arch Gen Psych*, **57**:925–41.

Sapolsky, R.M., Krey, L.C., & McEwen, B.S. (1986), 'The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis', *Endocr Rev*, **7**:284–301.

Sapolsky, R.M., Romero, L.M., & Munck, A.U. (2000), 'How do glucocorticoids influence stress-responses? Integrating permissive, suppressive, stimulatory, and preparative actions', *Endocrine Reviews*, **21**:55–73.

Sapolsky, R.M. Krey, L., & McEwen, B.S. (1983), 'Corticosterone receptors decline in a site-specific manner in the aged rat brain', *Brain Res*, **289**:235–40.

Sapolsky, R.M., Krey, L.C., & McEwen, B.S. (1984), 'Stress down-regulates corticosterone receptors in a site-specific manner in the brain', *Endocrinol*, **114**:287–92.

Sapolsky, R.M., Krey, L.C., & McEwen, B.S. (1986), 'The neuroendocrinology of stress and aging: The glucocorticoid cascade hypothesis', *Endocri Rev*, **7**:284–301.

Schmitt, L.H., Bradley, A.J., Kemper, C.M., Kitchener, D.J., Humphreys, W.F., & How, R.A. (1989), 'The demography and ecophysiology of *Dasyurus hallucatus* (Marsupialia: Dasyuridae) in the Mitchell Plateau Area, Kimberley, Western Australia', *J Zool Lond*, **217**:539–58.

Scully, J.L., & Otten, U. (1995), 'Glucocorticoids, neurotrophins and neurodegeneration', *J Steroid Biochem Molec Biol*, **52**:391–401.

Seckl, J.R., French, K.L., O'Donnell, D., Meaney, M.J., Nair, N,.P., Yates, C.M., & Fink, G. (1993), 'Glucocorticoid receptor gene expression is unaltered in hippocampal neurons in Alzheimer's disease', *Brain Res Mol Brain Res*, **18**:239–45.

Seckl, J.R., & Olsson, T. (1995), 'Glucocorticoid hypersecretion and the age impaired hippocampus: cause or effect?', *J Endocrinol*, **145**:201–11.

Scott, M.P. (1987), 'The effect of mating,and agonistic experience on adrenal function and mortality of male *Antechinus stuartii* (Marsupialia)', **68**:479–86.

Sernia, C., Bradley, A.J., & McDonald, I.R. (1979), 'High affinity binding of adrenocortical and gonadal steroids by plasma proteins of Australian marsupials', *Gen Comp Endocr*, **38**:496–503.

Sherwood, B.F., Rowlands, D.T., Hackle, D.B., & Le May, J.C. (1968), 'Bacterial endocarditis, glomerulonephritis and amyloidosis in the opossum (*Didelphis virginiana*)', *Am J Path*, **53**:115–26.

Soderquist, T.R. (1993), 'Maternal strategies of *Phascogale tapoatafa* (Marsupialia: Dasyuridae), I. Breeding seasonality and maternal investment', *Aust J Zool*, **41**:549–66.

Soderquist, T.R., & Ealey, L. (1994), 'Social interactions and mating strategies of a solitary carnivorous marsupial, *Phascogale tapoatafa*, in the wild', *Wildl Res*, **21**:527–42.

Squire, L. (1987), *Memory & Brain*, New York, Oxford Univ. Press.

Steins-Behrens, B.A., & Sapolsky, R.M. (1992), 'Stress, glucocorticoids and aging', *Aging Clin Exp Res*, **4**:197–210.

Stemmerman, G.N., & Hayashi, T. (1969), 'A survey of the normal and morbid anatomy of the Hawaiian feral mongoose', *Am J Path*, **55**:67a–68a.

Sterling, P., & Eyer, J. (1981), 'Biological basis of stress-related mortality', *Soc Sci Med E*, **15**:3–42.

Sumner, J., & Dickman, C.R. (1998), 'Distribution and identity of speciied in the *Antechinus stuartii – A. flavipes* group (Marsupialia: Dasuyuridae) in south-eastern Australia', *Aust J Zool*, **46**:27–41.

Svanes, K., Varhaug, J.E., Dzienis, H., & Gronbech, J.E. (1984), 'Gastric mucosal blood flow related to acute mucosal damage', *Scand J Gastroenterol*, **19**:62–6.

Toftegaard, C.L. (1999), 'Morphological and endocrine correlates of chemical communication during the life history of *Antechinus stuartii* (Macleay)', PhD thesis, The University of Queensland, Brisbane, Australia.

Toftegaard, C.L., Moore, C., & Bradley, A.J. (1999), 'Chemical characterisation of urinary pheromones in the brown antechinus, *Antechinus stuartii* (Marsupialia: Dasyuridae)', *J Chemical Ecology*, **25**:527–35.

Toftegaard, C.L., McMahon, K.L., Galloway, G.J., & Bradley, A.J. (2002). 'Processing of urinary pheromones in *Antechinus stuartii* (Marsupialia: Dasyuridae): Functional magnetic resonance imaging of the brain', *J Mammal*, **83**:71–80.

Tyndale-Biscoe, H., & Renfree, M. (1987), *Reproductive Physiology of Marsupials*, Cambridge University Press, Cambridge.

Van Dyck, S., & Crowther, M.S. (2000), 'Reassessment of Northern representatives of the *Antechinus stuartii* complex (Marsupialia; Dasyuridae): *A. subrtopicus* sp.nov., & *A. adustus* new status', *Mem Qld Museum*, **45**:611–35.

Van Eekelen, J.A., & De Kloet, E.R. (1992), 'Co-localization of brain corticosteroid receptors in the rat hippocampus'. *Prog Histochem Cytochem*, **26**:250–8.

Von Holst (1972), 'Renal failure as a cause of death in *Tupaia belangeri*. exposed to persistent social stress', *J Comp Physiol*, **78**:236–73.

Von Holst (1986), 'Psychosocial stress and its pathophysiological efects in tree shrews (*Tupaia belangeri*)', in *Biological and Psychological Factors in Cardiovascular Disease* (eds. T.H. Schmidt, T.M. Dembroski, & G. Blumchen), Springer-Verlag, Berlin.

Wexler, B.C., & Greenberg, B.P. (1978), 'Pathophysiological differences between paired and communal breeding of male and female Sprague-Dawley rats', *Circulation Research*, **42**:126–35.

Williams, R., & Williams, A. (1982), 'The life cycle of *Antechinus swainsonii* (Dasyuridae: Marsupialia)', in *Carnivorous Marsupials* (ed. M. Archer), pp. 89–95, Royal Zoological Society of New South Wales, Sydney.

Wilson, M. (1985), 'Hippocampal inhibition of the pituitary-adrenocortical response to stress', in *Psychological and Physiological Interactions in Response to Stress* (ed. S. Birchfield), Academic Press, New York.

Woollard, P. (1971), 'Differential mortality of *Antechinus stuartii* (Macleay): nitrogen balance and somatic changes', *Aust J Zool*, **19**:347–53.

Woolley, P.A. (1966), 'Reproduction in *Antechinus spp.*, & other dasyurid marsupials', *Symp Zool Soc Lond*, **15**:281–94.

Woolley, P.A. (1991), 'Reproductive pattern of captive Boullanger Island dibblers, *Parantechinus apicalis* (Marsupialia: Dasyuridae)', *Wildlife Research*, **18**:157–63.